

Practice Laminar high Resolution fMRI Analysis

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Peters Lab pipeline

Based on Oliver Warrington PhD Thesis

Communication of perceptual predictions from the hippocampus to the deep layers of the parahippocampal cortex

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Anatomical Preprocessing

Cortical Surface Reconstruction and Coregistration:

1. The gradient-echo image acquired at the later inversion time (INV2) volume was bias-corrected and segmented with unified segmentation method in SPM.
2. The CSF, bone, non-brain tissue and background tissue classes were combined with a threshold of > 0.5 and then inverted to create a brainmask.
3. The combined uniform (UNI) volume was denoised with the mp2rage toolbox for SPM12 with threshold of 6
4. The denoised UNI image was then skull-stripped using the INV2-derived brain mask.
5. The pial and white matter surfaces were reconstructed with CAT (input: The skull-stripped UNI)
6. The local intensity corrected output of cat12 was supplied to recon-all with the hires pipeline and samseg segmentation.
7. The cortical surfaces were then coregistered to the mean functional image using the OpenFmriAnalysis toolbox.

Anatomical Preprocessing

Cortical Surface Reconstruction and Co-registration:

8. First, the MT-weighted whole-brain EPI scan was coregistered and resliced with a rigid-body transformation to the mean functional using FSL FLIRT
9. The whole-brain EPI was then used as the target for surface coregistration due to the improved grey/white matter contrast imparted by the MT-weighting.
10. A rigid-body transformation between the UNI image and the whole-brain EPI was calculated using SPM12.
11. This transformation was then applied to the coordinates of each vertex comprising the pial and white matter surfaces, resulting in cortical surfaces in the functional space of each participant. These surfaces were then used to define the cortical layers.

Anatomical Preprocessing

Definition of the cortical layers:

1. First, The grey matter was divided into three equivolume layers using the level set method (described in detail in [66] and [67])
2. We calculated two intermediate surfaces between the WM and pial boundaries, yielding three GM layers (deep, middle, and superficial).
3. Based on these surfaces, we calculated four signed distance functions (SDF), containing for each functional voxel its distance to the boundaries between the five cortical compartments (WM, CSF, and the 3 GM layers). This set of SDFs (or “level set”) allowed the calculation of the distribution of each voxel’s volume over the five compartments [66].
4. For each cortical ROI (see below), voxels were assigned to one of the three GM layers only if >50% of its volume resided within that layer.

66. Van Mourik T, Van Der Eerden JPJM, Bazin P-L, Norris DG. Laminar signal extraction over extended cortical areas by means of a spatial GLM. Bernhardt BC, editor. PLOS ONE. 2019;14: e0212493. doi:10.1371/ journal.pone.0212493

67. Kok P, Bains LJ, van Mourik T, Norris DG, de Lange FP. Selective Activation of the Deep Layers of the Human Primary Visual Cortex by Top-Down Feedback. Current Biology. 2016;26: 371–376. doi:10.1016/ j.cub.2015.12.038

Anatomical Preprocessing

Regions of interest:

1. The medial temporal lobe was segmented using the automatic segmentation of hippocampal subfields (ASHS) [22] machine learning toolbox in conjunction with a database of manual 7T medial temporal lobe segmentations from a separate set of participants.
2. The hippocampal subfields and MTL cortices were defined on the anatomy of the T1- and T2-weighted images. The T1-weighted image was the denoised UNI volume and the T2-weighted image comprised the average of two high-resolution T2-weighted scans which were combined and denoised using the Structural Averaging Toolbox.
3. All regions segmented with ASHS were coregistered to the mean functional image of each participant using FSL FLIRT.

fMRI Preprocessing

1. Functional volumes denoised with NORDIC algorithm.
2. low signal slices removed.
3. Distortion correction using FSL Topup and SPM12 realign and unwarp.
4. GLM

Functional

NORDIC

SPM

Denoise
Distortion
correction
Motion
correction

Anatomical

SPM

Freesurfer

CAT

ASHS

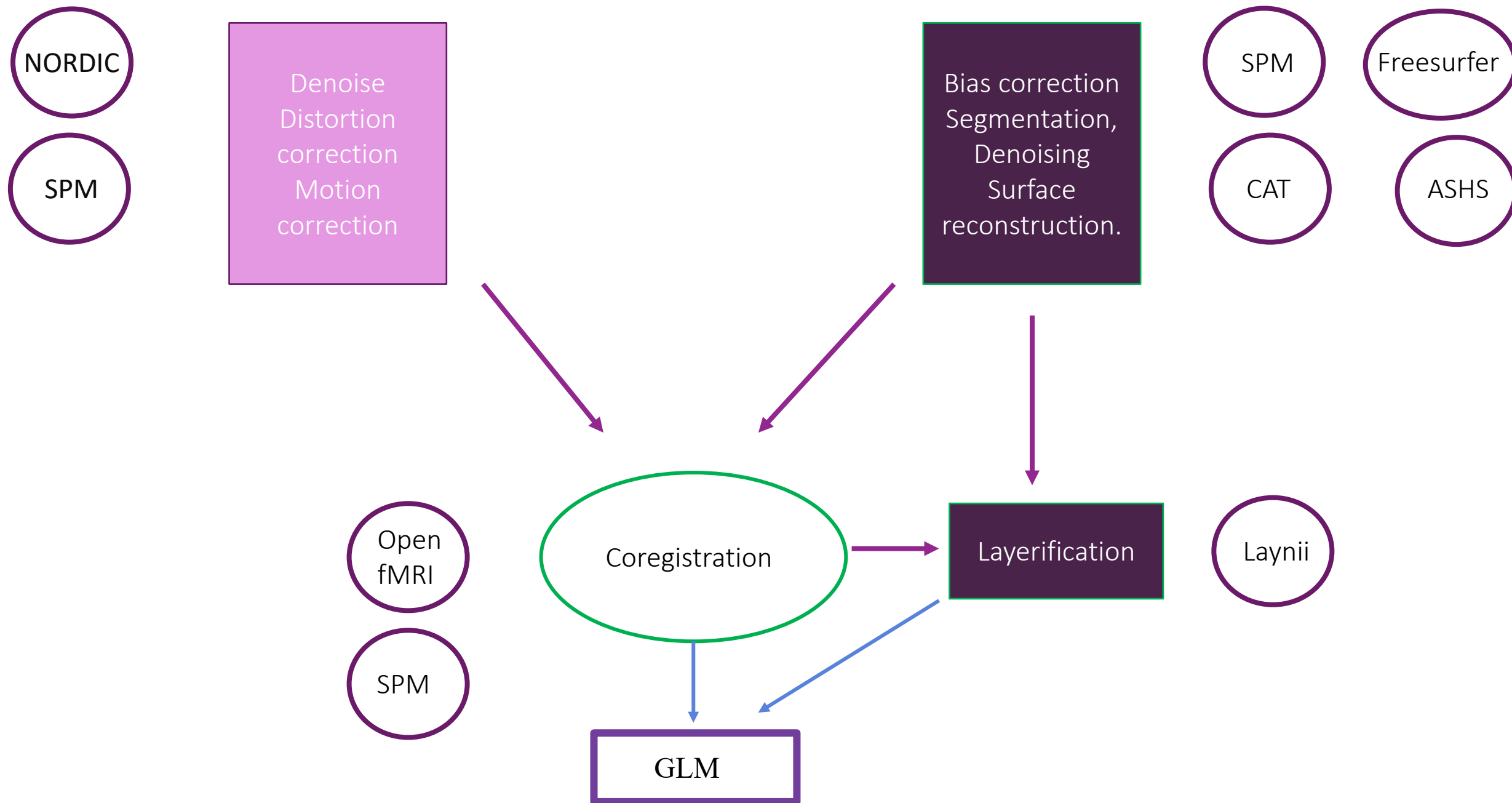
Bias correction
Segmentation,
Denoising
Surface
reconstruction.

Coregistration

Layerification

Laynii

GLM



Functional

NORDIC ✓

SPM ✓

FSL
Topup ✗

Denoise
Distortion
correction
Motion
correction

What I can do with my
near dead laptop, and
provided data!

Anatomical

Bias correction
Segmentation,
Denoising
Surface
reconstruction.

SPM ✓

Freesurfer ✓

CAT ✓

ASHS ✗

✗ Open
fMRI

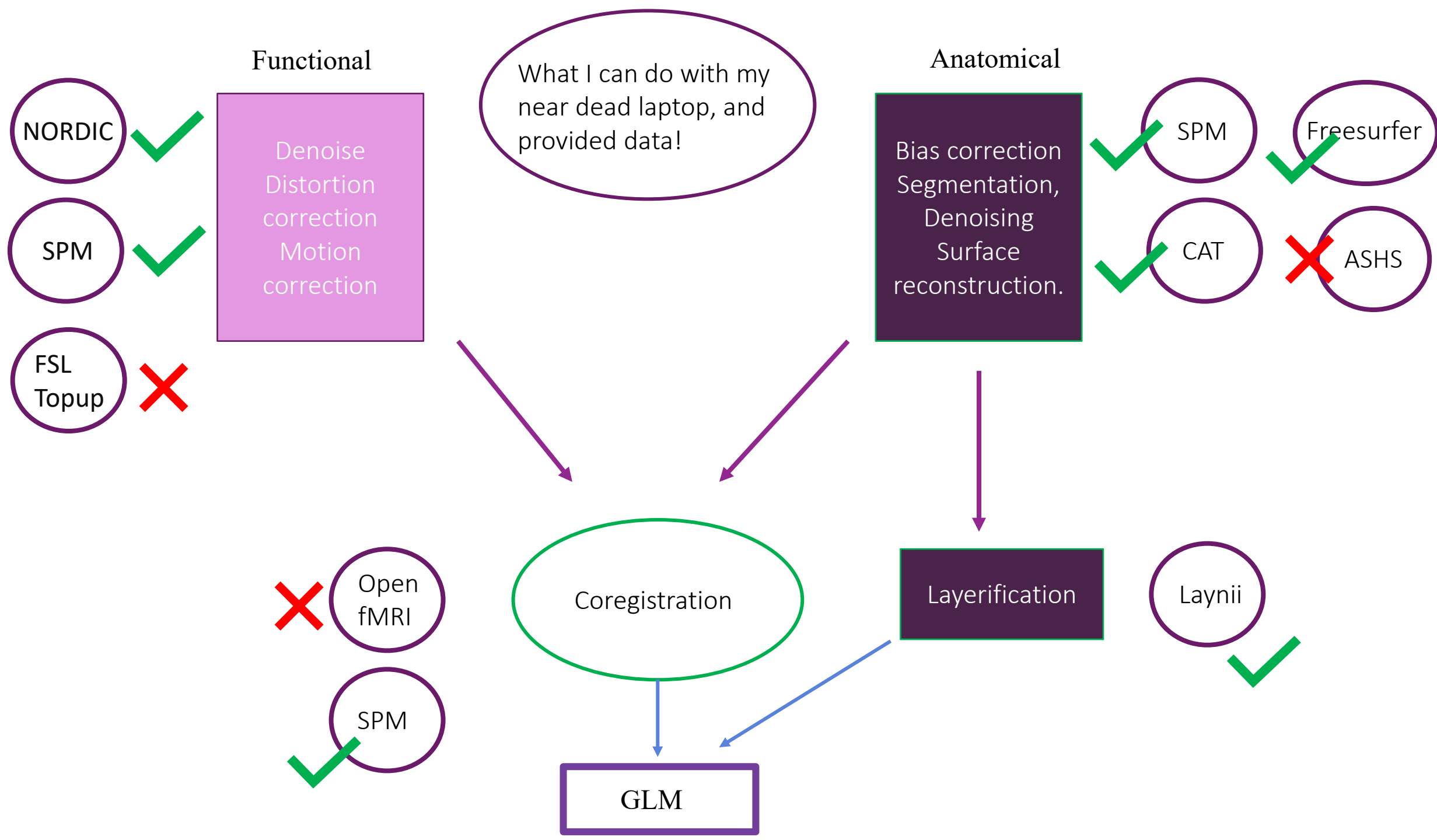
✓ SPM

Coregistration

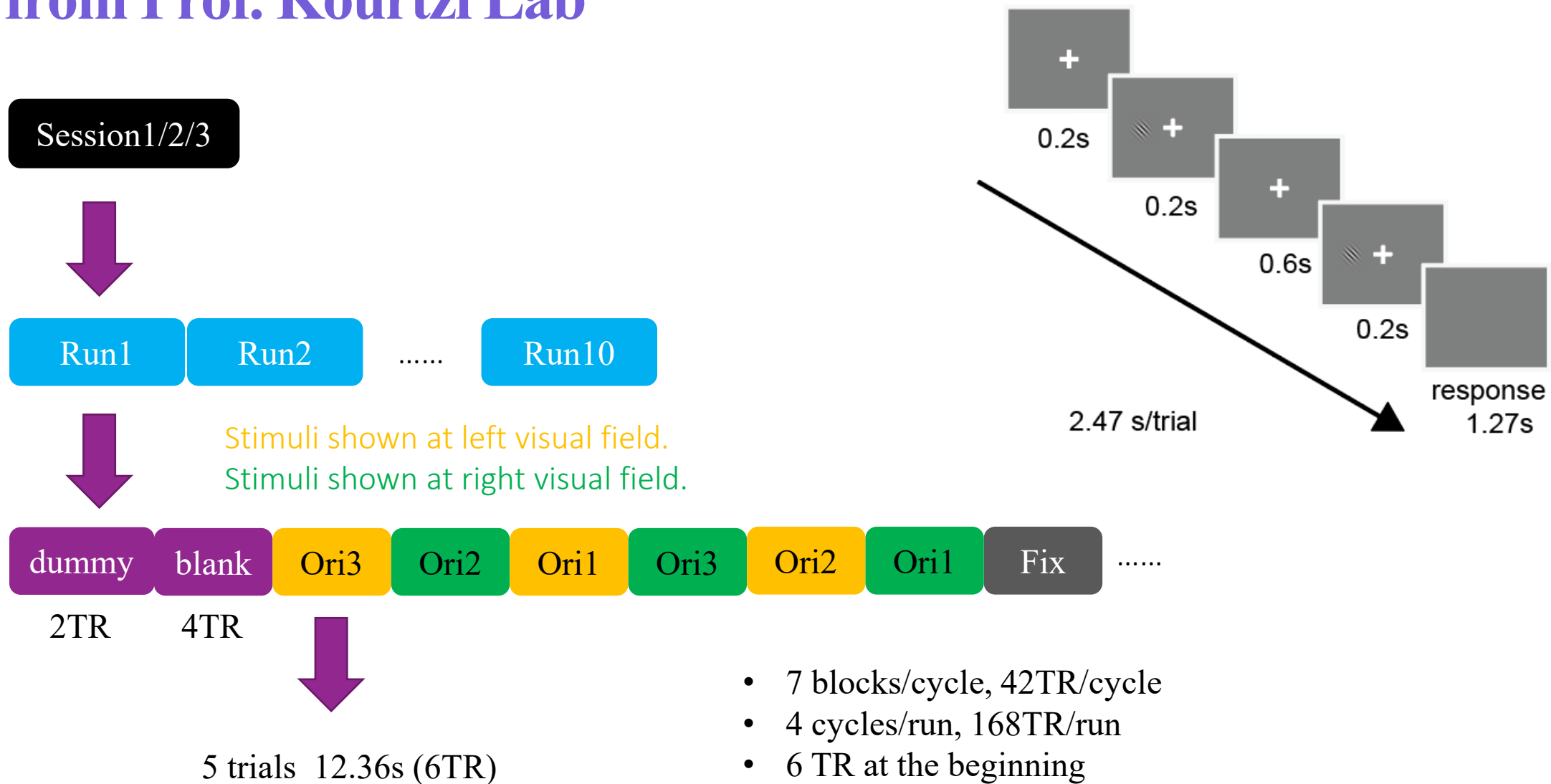
Layerification

Laynii ✓

GLM



Data from Prof. Kourtzi Lab

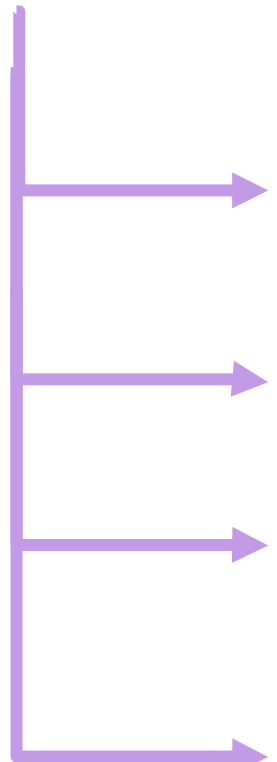


- 7 blocks/cycle, 42TR/cycle
- 4 cycles/run, 168TR/run
- 6 TR at the beginning
- In total, 174TR/run

Data from Prof. Kourtzi Lab

	Session 1 (PL-Pre)	Session 2 (PL-Post)	Session 3 (Control)
Before EPI	0.65iso anatomical, ~35 mins	fast anatomical, ~30 mins	fast anatomical, ~30 mins
Task Runs	FDL (7.5 mins) * 10 ~80mins	FDL (7.5 mins) * 10 ~80mins	FDL (7.5 mins) * 10 ~80mins
Other runs	Resting State ~10min	Resting State ~10min	Retinotopy ~10min
In total	120 mins	120 mins	120 mins

Segmentation

- 
1. Pre-processing (skull-Stripped UNI)
 2. MP2RAGE data to Free Surfer
 3. MP2RAGE data to CAT12
 4. Manual Segmentation with ITK-SNAP > create rim file (after co-registration)

MP2RAGE data to CAT12

SPM>CAT12>preprocessing>Segment>Batch(Surface and Thickness estimation: Yes, Deformation: Forward, Atlas: Yes)

CAT-Segmentation: ..analysis\training dataset_kurtzi\Shared\anatomical_highRes\zk18w7_042_brain.nii

Version: OS / Matlab / SPM12 / CAT12 / seg:

WIN / 9.13 / 7771 / 12.9 (2577) / [1639](#)

Tissue Probability Map:

\color[rgb]{0 0 0}..ers\ASUS\Desktop\Apps\spm12\tpm\TPM.nii

Optimized Shooting Registration to:

..s\MNI152Nlin2009cAsym\Template_0_GS.nii

affreg / APP / setCOM

mni / default / COM

biasstr

medium

LAS strength / Skull-Stripping:

medium / APRG

WMH Correction / Int. Res.:

(WMH=WM) / optimal(1.00 0.30)

Voxel resolution (original > internal > PBT; vox):

0.65x0.65x0.65 > 0.65x0.65x0.65 > 0.50³ mm³; 1.50³ mm³

Image and Preprocessing Quality:

Resolution:

92.00% (A-)

Noise:

100.00% (A+)

Bias:

74.72% (C)

Weighted average (IQR):

93.08% (A-)

Mean surface Euler number:

40

Defect area:

1.26%

Processing time:

52:38 min

Volumes:

CSF GM WM

Absolute volume:

220 593 478 cm³

Relative volume:

17.0 45.9 37.0 %

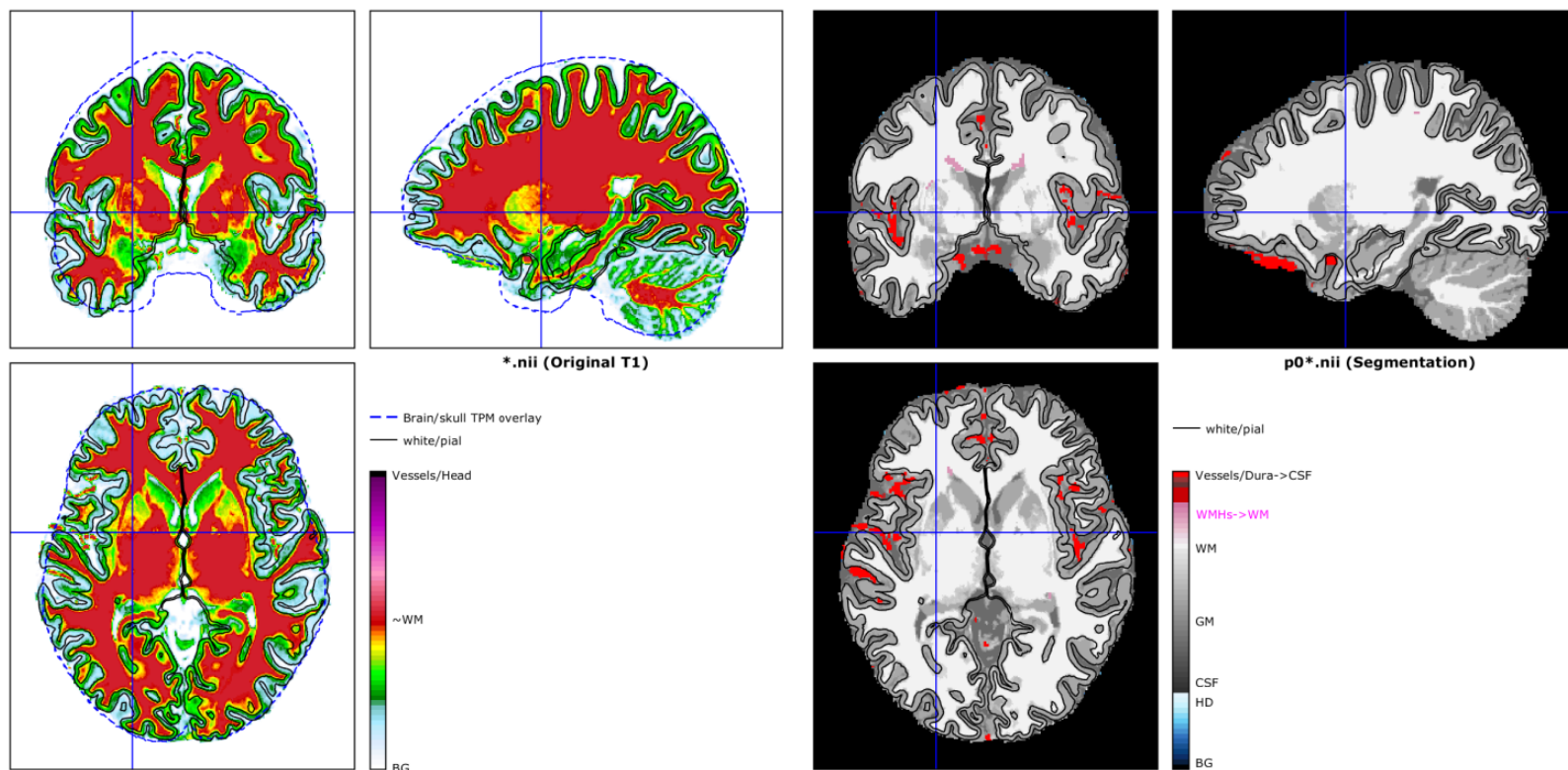
TIV:

1290 cm³

Thickness:

1.95±0.52 mm

MP2RAGE data to CAT12: for surfaces



FreeSurfer 7.1.1: for segmentation

Code:

```
recon-all -subject test-subject -i ~/ zk18w7_042_brain.nii.gz -all
```

For outputs:

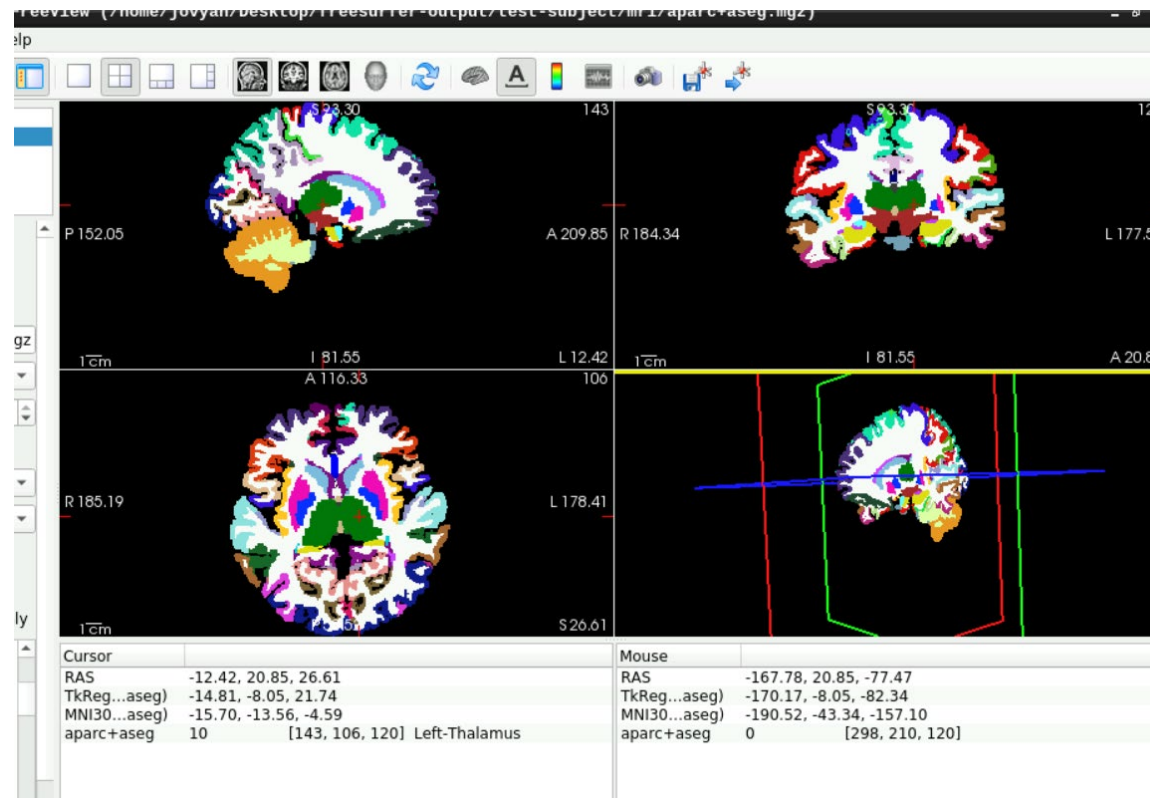
```
mri_label2vol -seg ribbon.mgz -temp rawavg.mgz -o ribbon-in-rawavg.mgz -regheader  
ribbon.mgz
```

```
mri_convert ribbon-in-rawavg.mgz ribbon-in-rawavg.nii
```

```
mri_convert rawavg.mgz rawavg.nii
```

FreeSurfer 7.1.1

Aparak + aseg



Co-registration with SPM (pial and white matter surface)

```
spm('defaults', 'FMRI');  
spm_jobman('initcfg');  
  
% Paths  
mean_func = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/meanufunctional_data.nii'; % mean functional image  
t1_image = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/ribbon-in-rawavg.nii'; % segmented output from freesurfer  
% Coregister T1 to mean functional image  
  
matlabbatch{1}.spm.spatial.coreg.estimate.ref = {mean_func};  
matlabbatch{1}.spm.spatial.coreg.estimate.source = {t1_image};  
  
spm_jobman('run', matlabbatch);  
  
% Load transformation matrix  
transformation_matrix = spm_get_space(t1_image);  
  
% Surface files  
lh_pial = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/lh.pial.zk18w7_042_brain.gii';  
rh_pial = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/rh.pial.zk18w7_042_brain.gii';  
lh_white = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/lh.white.zk18w7_042_brain.gii';  
rh_white = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/rh.white.zk18w7_042_brain.gii';
```

Co-registration with SPM (pial and white matter surface)

```
% Apply transformation to surfaces
```

```
apply_transformation_to_surface(lh_pial , transformation_matrix);  
apply_transformation_to_surface(rh_pial , transformation_matrix);  
apply_transformation_to_surface(lh_white, transformation_matrix);  
apply_transformation_to_surface(rh_white, transformation_matrix);
```

```
function apply_transformation_to_surface(surface_file, transformation_matrix)
```

```
% Load the surface file
```

```
surf = gifti(surface_file);
```

```
% Apply the transformation matrix to the vertices
```

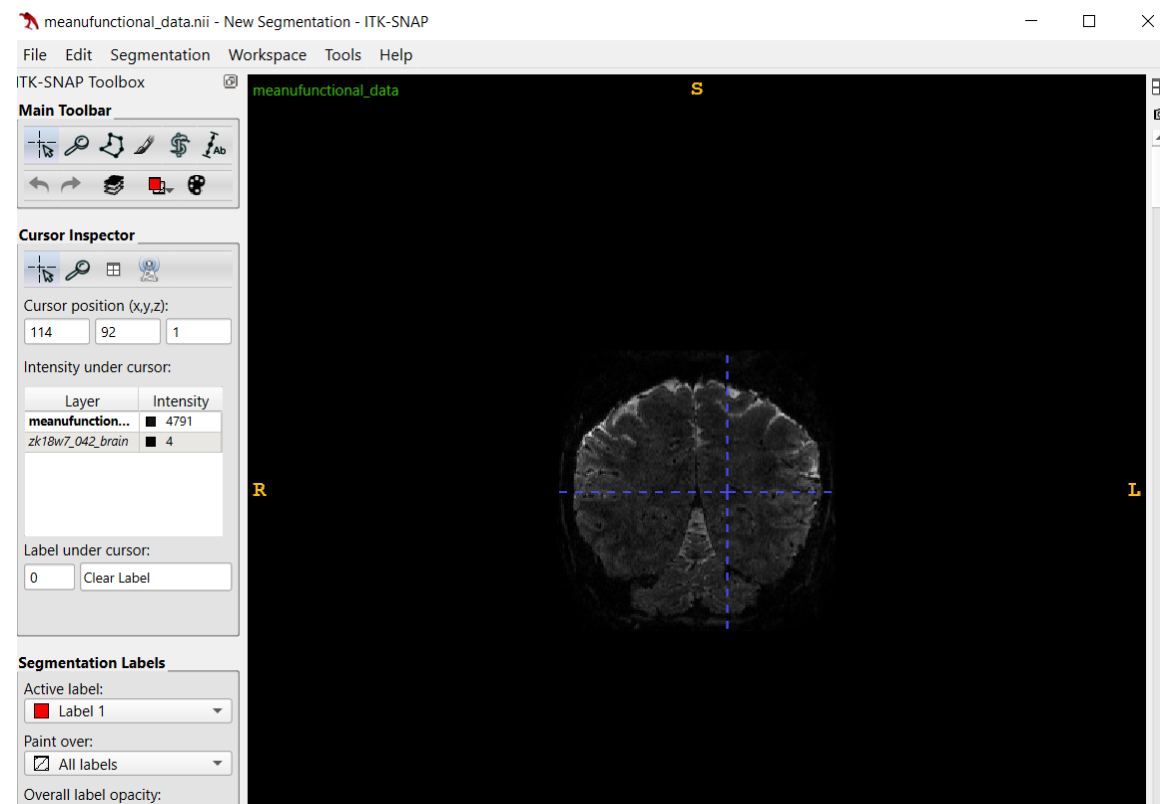
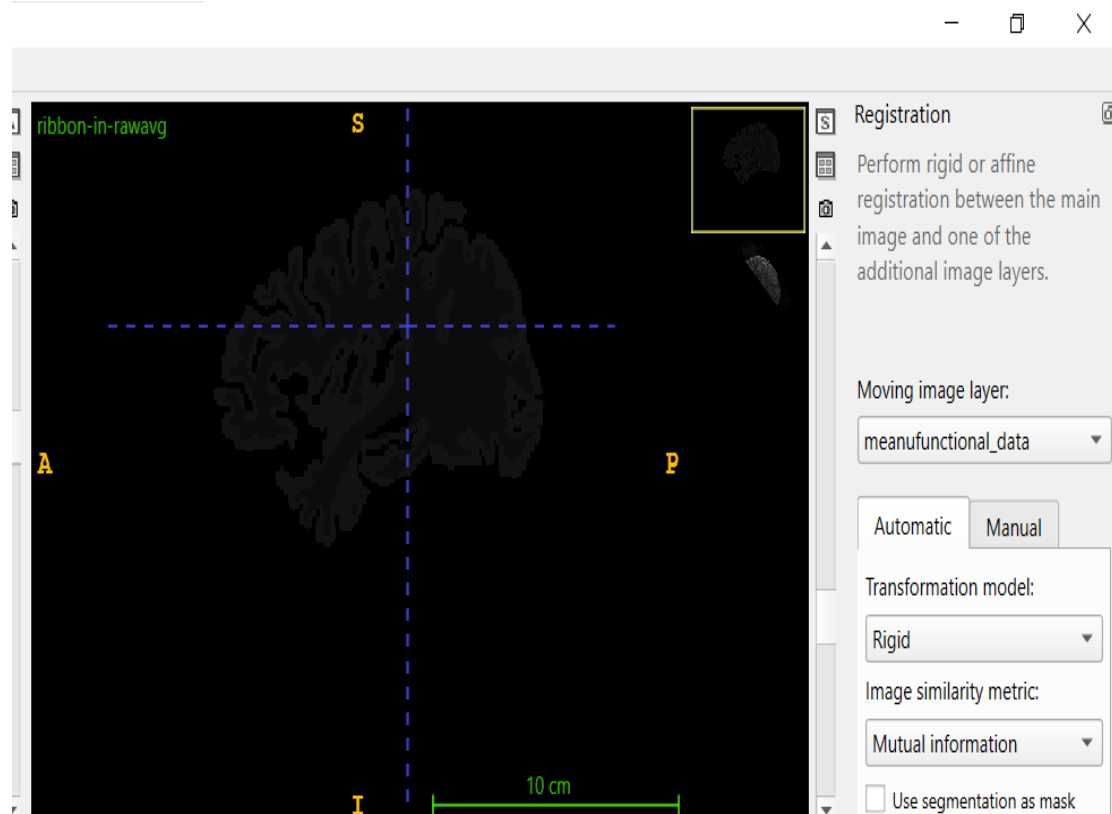
```
surf.vertices = (transformation_matrix(1:3, 1:3) * surf.vertices' + transformation_matrix(1:3, 4))';
```

```
% Save the transformed surface
```

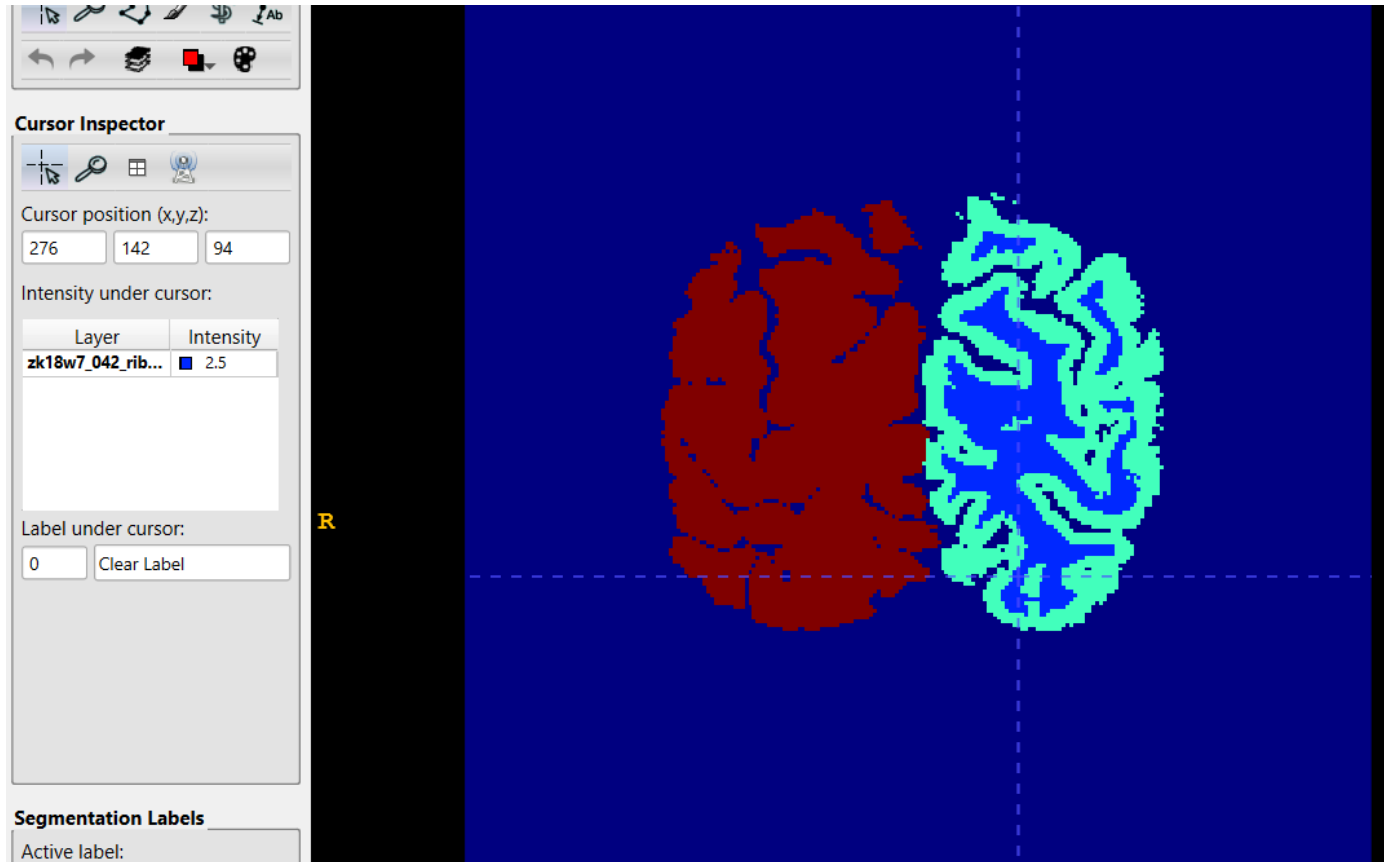
```
save(surf, surface_file);
```

```
end
```

Check co-registration manually with ITK-SNAP

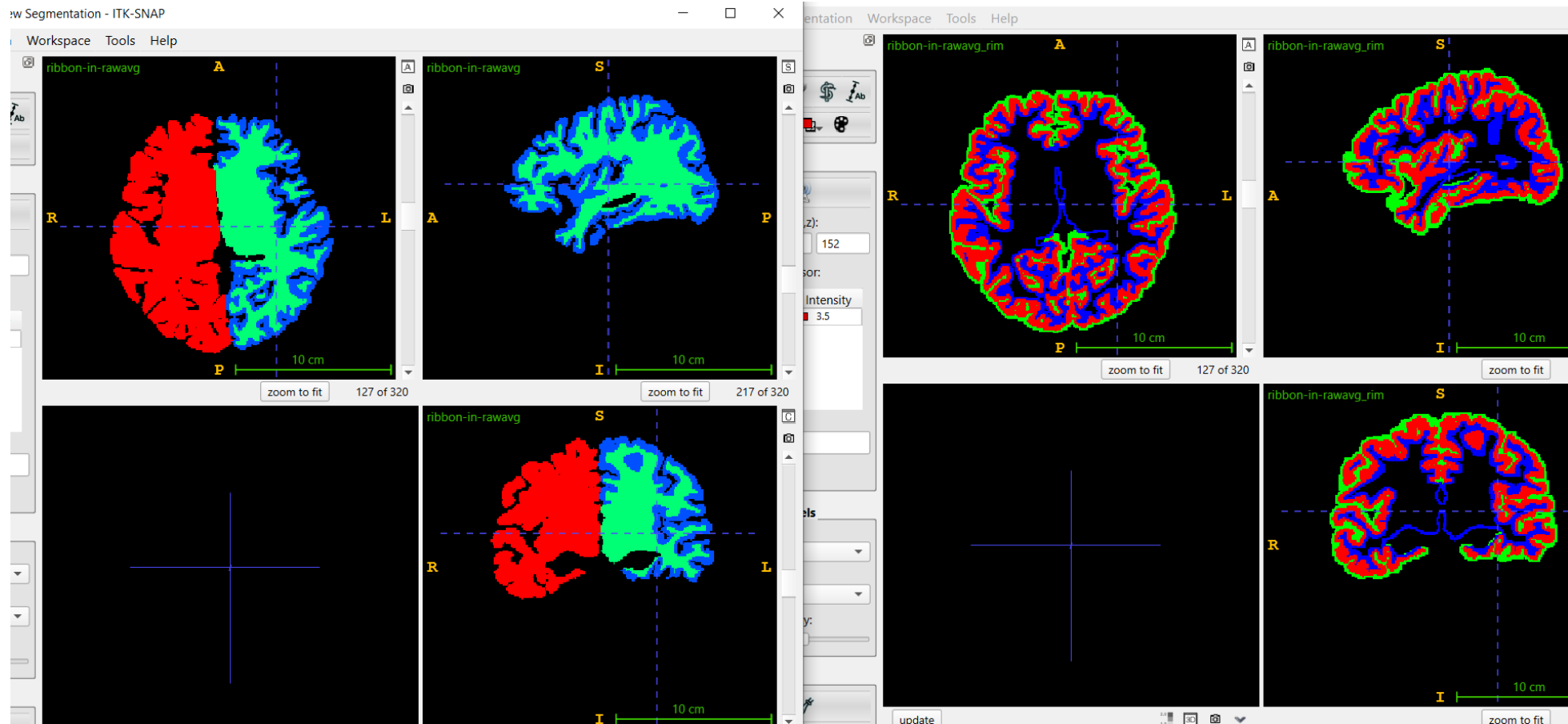


Further Manual Segmentation with ITK-SNAP



Change Labels (transfer free surfer output to rim file for laynii)

Code: https://github.com/ofgulban/LAYNII_extras/blob/38e607edbd6601b5893e519af3f791059fbe190d/demo_freesurfer_segmentation_to_rim/freesurfer_segmentation_to_rim.py



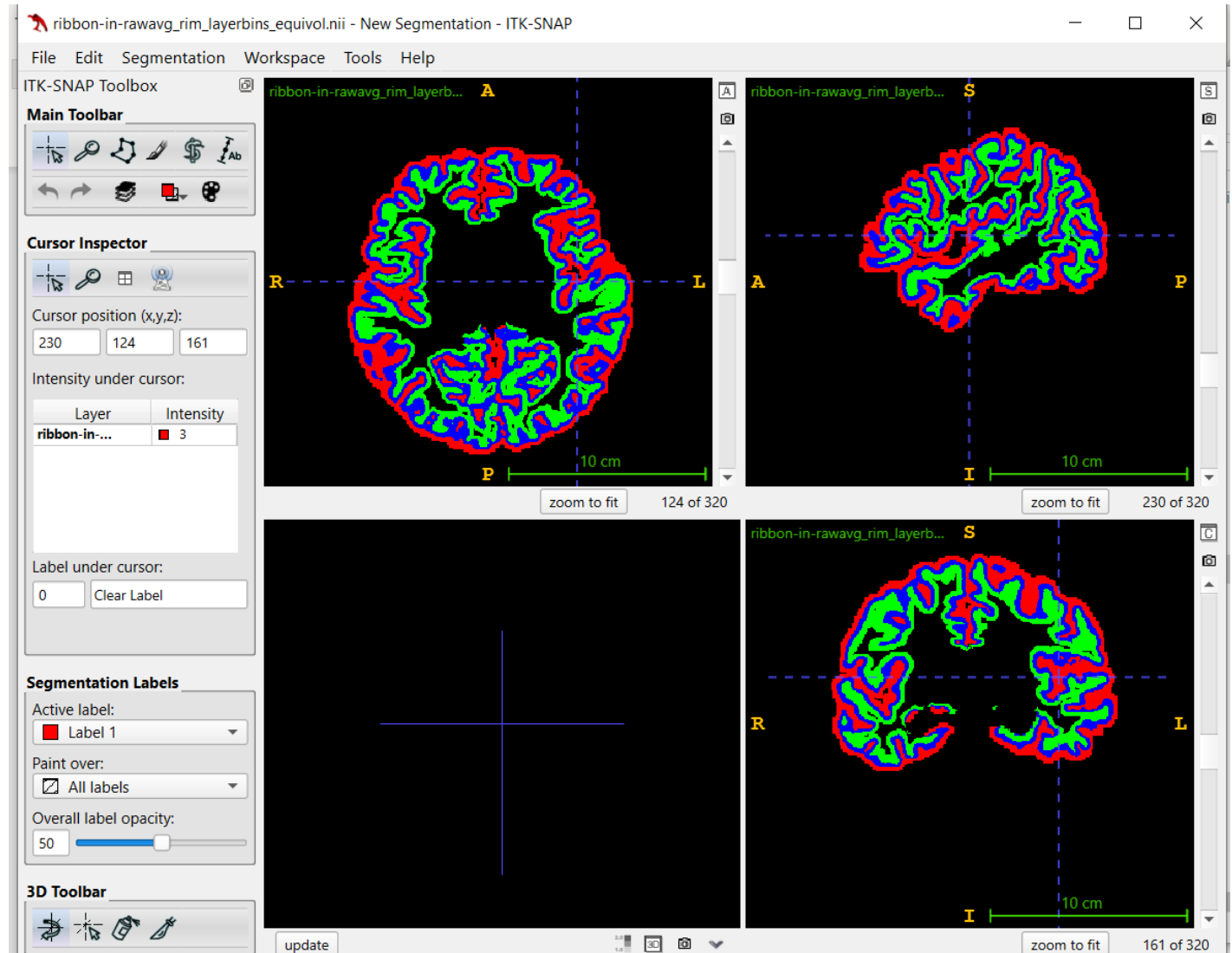
Segmentation with ASHS

I couldn't find a suitable atlas for my data. Lets keep going with rim file.

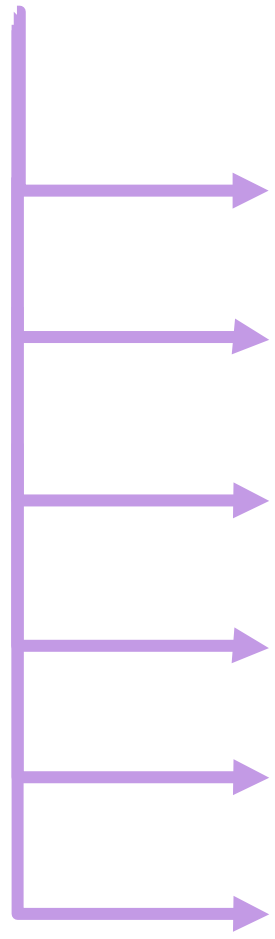
Defining Cortical Layers in LYNII (level set method)

Code :

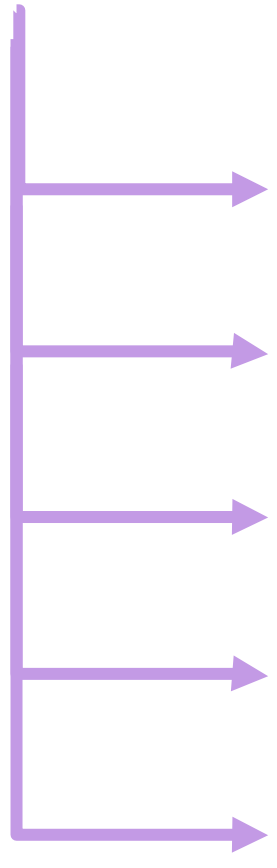
```
LN2_LAYERS -rim ribbon-  
in-rawavg_rim.nii -  
nr_layers 3 -  
equal_counts -equivol
```



Functional data

- 
1. Create 4D nifty with SPM
 2. Creating mean image with SPM
 3. realign and unwarp for motion and distortion correction (not possible; I couldn't use ph hp phase)
 4. Quality check with Lynii (tSNR)
 5. Using NORDIC for denoising
 6. Quality check again

Functional data

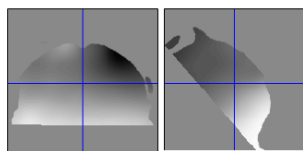
- 
- 7. Slice time correction (skipped)
 - 8. Co-registration with OpenFmriAnalysis toolbox: (not possible)
 - 9. Co-registration with SPM toolbox and ITK-SNAP
 - 10. Create V1 mask after Layerification
 - 11. Activation within layers of V1

Realign and unwrap for motion and distortion correction

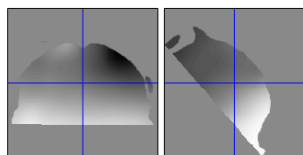
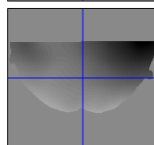
Realign and unwrap (I have no such a phase map file)



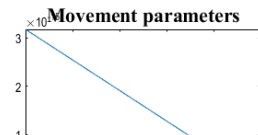
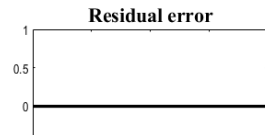
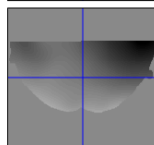
Estimation of EPI deformation fields



Derivative w.r.t. pitch



Derivative w.r.t. roll



Realign (Estimate and resliced)

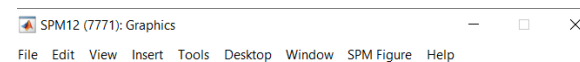
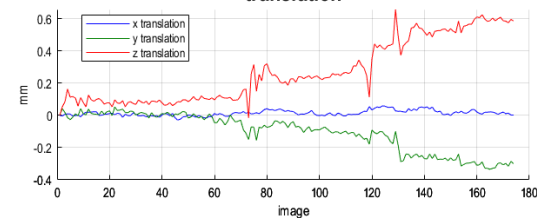


Image realignment

```
1 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
2 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
3 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
4 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
5 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
6 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
7 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
8 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
9 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
10 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
11 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
12 D:\high field fMRI analysis\training dataset_kurtzi\Shared\2_functional_highRes\functionz
..... etc
```

translation



rotation



NORDIC denoising

Code:

v1.1 from https://github.com/SteenMoeller/NORDIC_Raw/releases

```
ARG.NORDIC=1;  
RG.noise_volume_last = 0;  
ARG.magnitude_only=1;  
%ARG.kernel_size_PCA = [28, 28, 1];  
fn_magn_in= 'rfunctional_data';  
fn_phase_in=fn_magn_in;  
fn_out=['NORDIC_' fn_magn_in];  
NIFTI_NORDIC(fn_magn_in,fn_phase_in,fn_out,ARG)
```

Quality Check (slice time correction skipped)

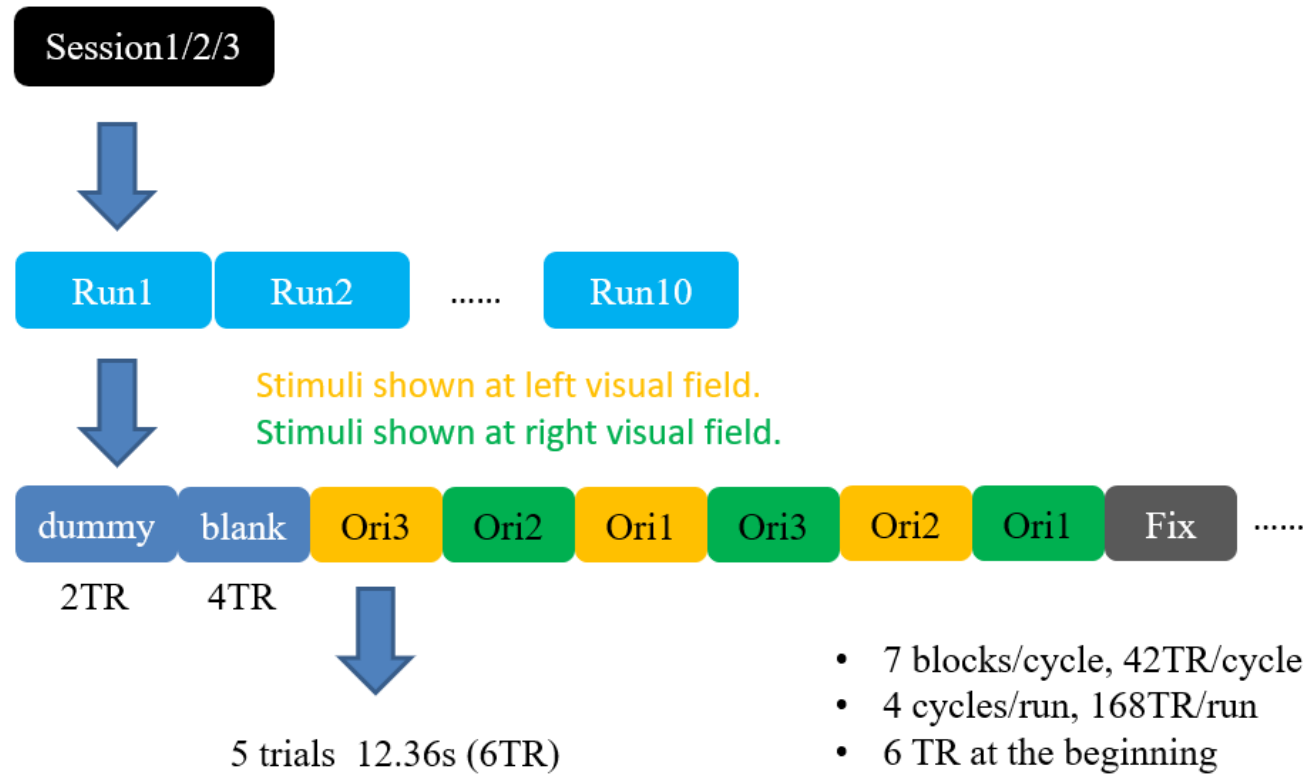
I want to see changes after NORDIC denoising

Code:

```
1.   LN_SKEW -input NORDIC_rfunctional_data.nii
2.   LN_SKEW -input rfunctional_data.nii
3.   GLM with Afni:
3dDeconvolve -overwrite -jobs 16 -polort a -input NORDIC_rfunctional_data.nii \
    -num_stims 3 \
    -TR_times 2.06 \
    -stim_times 1 '1D: 49.44 111.24 197.76 309' 'UBLOCK(12.36,1)' -stim_label 1 Task1 \
    -stim_times 2 '1D: 24.72 160.68 247.2 284.28' 'UBLOCK(12.36,1)' -stim_label 2 Task2 \
    -stim_times 3 '1D: 74.16 135.96 222.48 333.72' 'UBLOCK(12.36,1)' -stim_label 3 Task3 \
    -tout \
    -iresp 1 HRF_BOLD.nii \
    -x1D MODEL_wm \
    -bucket STATS.nii
```

participant's response (orientation 1-2-3), I just model for right hemisphere

GLM (onset, duration)



- 7 blocks/cycle, 42TR/cycle
- 4 cycles/run, 168TR/run
- 6 TR at the beginning
- In total, 174TR/run

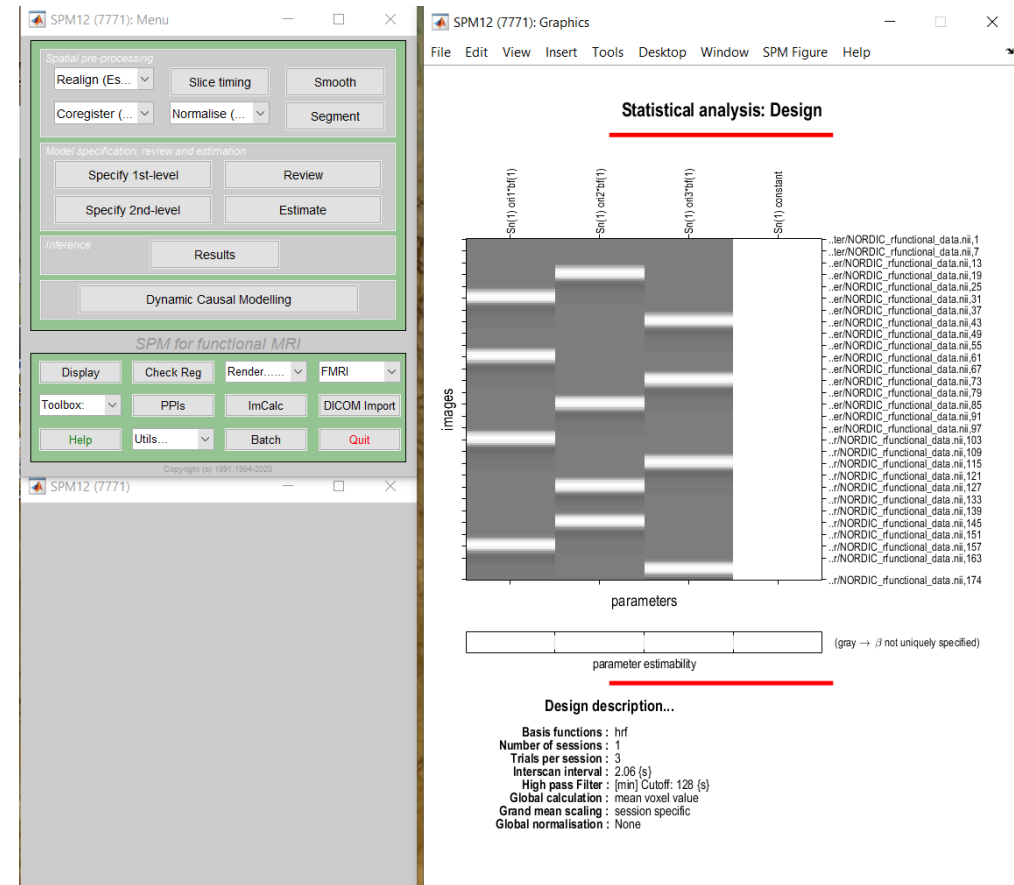
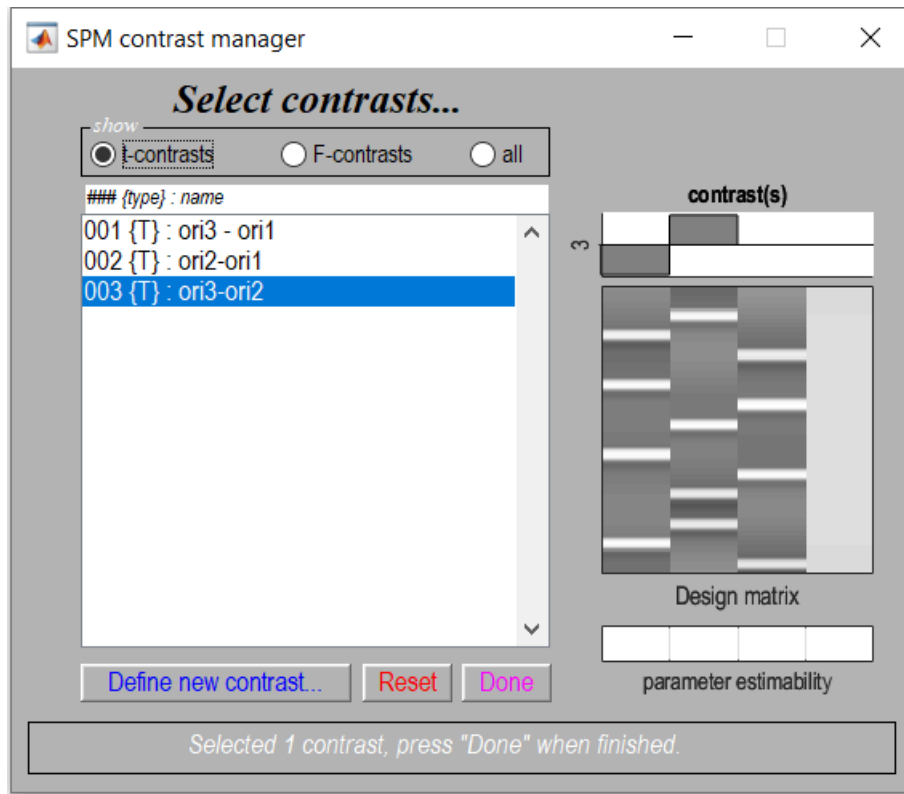
Right = 2

Ori1	49.44	111.24	197.76	309
Ori2	24.72	160.68	247.2	284.28
Ori3	74.16	135.96	222.48	333.72

Left = 1

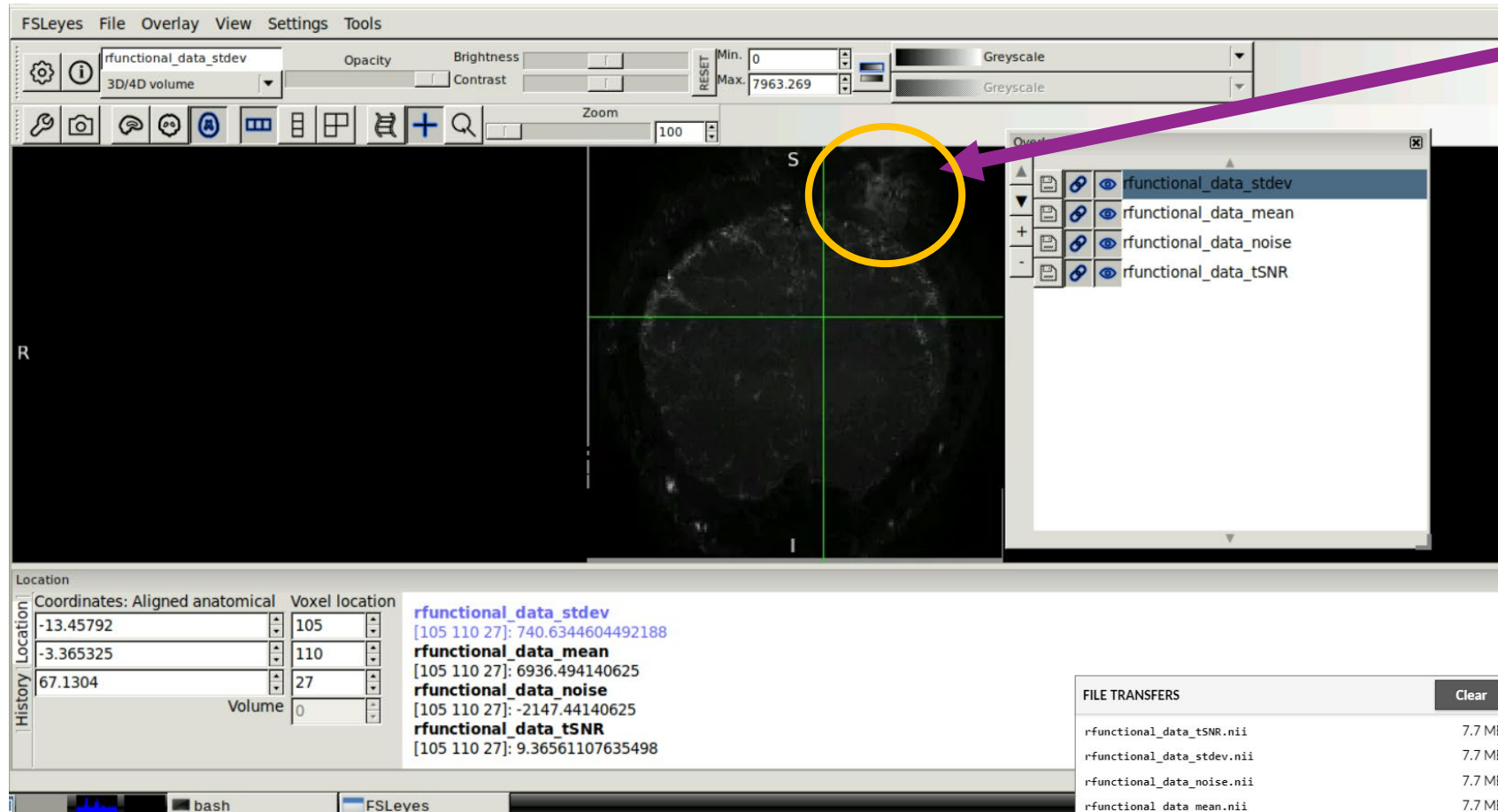
Ori1	12.36	123.6	234.84	296.64
Ori2	37.08	148.32	185.4	271.92
Ori3	61.8	98.88	210.12	321.36

GLM with SPM



Quality Check (no NORDIC)

Like ghost in stdev



FSLeves File Overlay View Settings Tools

rfunctional_data_stdev 3D/4D volume Opacity Brightness Contrast Min. 0 Max. 7963.269 Greyscale Greyscale

Zoom 100

R S I

rfunctional_data_stdev
rfunctional_data_mean
rfunctional_data_noise
rfunctional_data_tSNR

Location

Coordinates: Aligned anatomical	Voxel location
-13.45792	105
-3.365325	110
67.1304	27

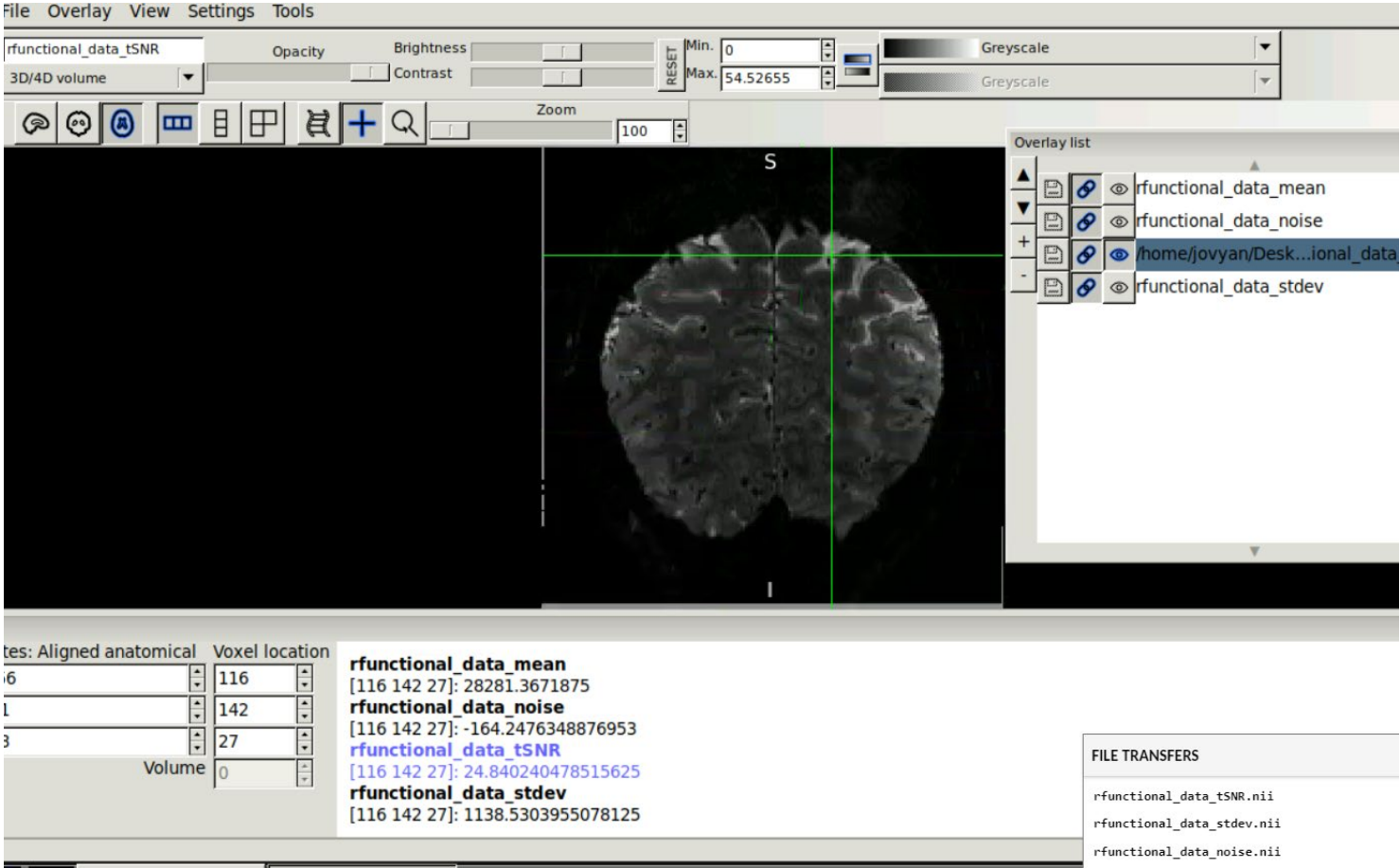
Volume 0

rfunctional_data_stdev
[105 110 27]: 740.6344604492188
rfunctional_data_mean
[105 110 27]: 6936.494140625
rfunctional_data_noise
[105 110 27]: -2147.44140625
rfunctional_data_tSNR
[105 110 27]: 9.36561107635498

FILE TRANSFERS		Clear
rfunctional_data_tSNR.nii	7.7 MB	
rfunctional_data_stdev.nii	7.7 MB	
rfunctional_data_noise.nii	7.7 MB	
rfunctional_data_mean.nii	7.7 MB	

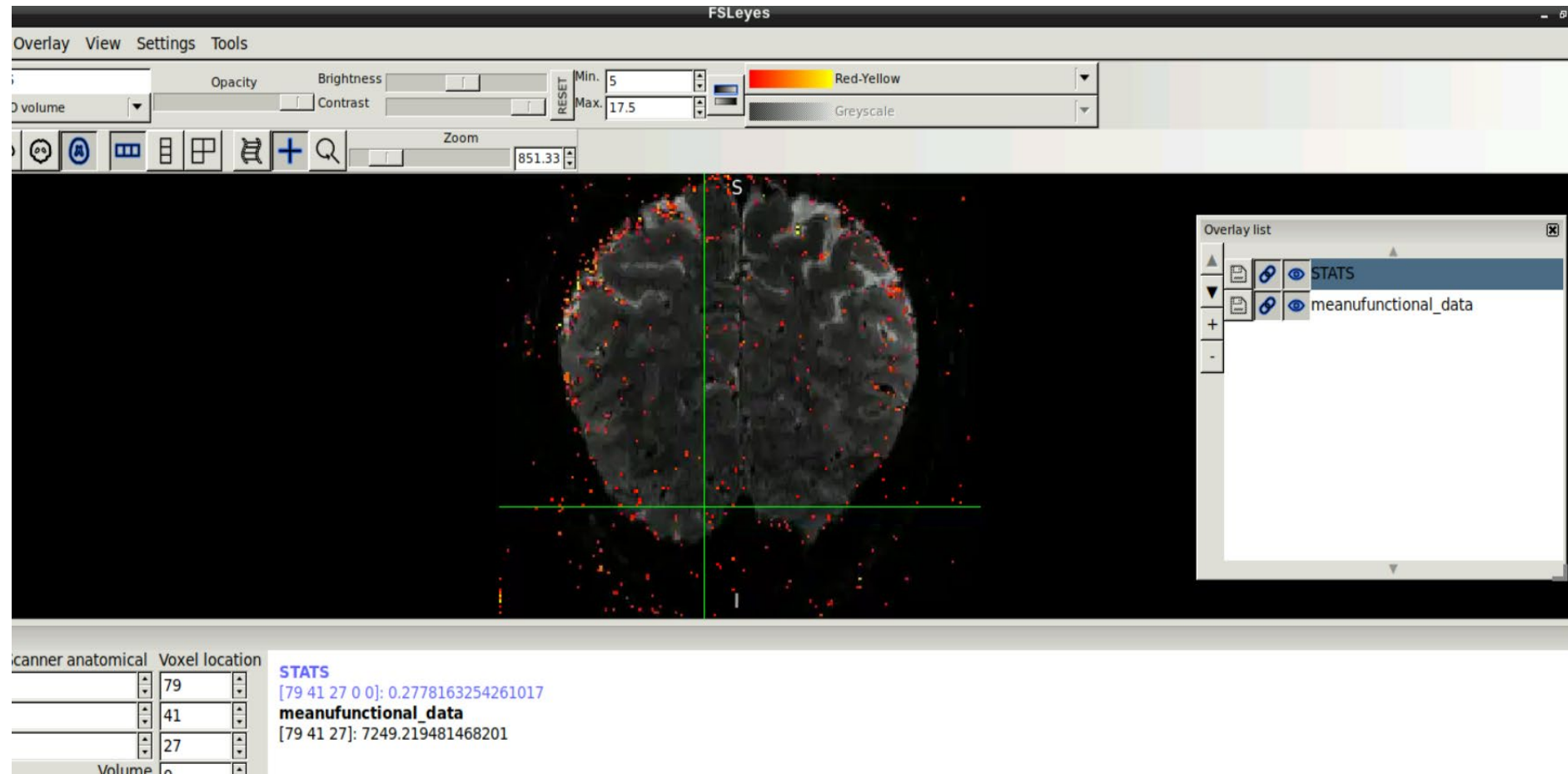
bash FSLeves

Quality Check (no NORDIC) tSNR



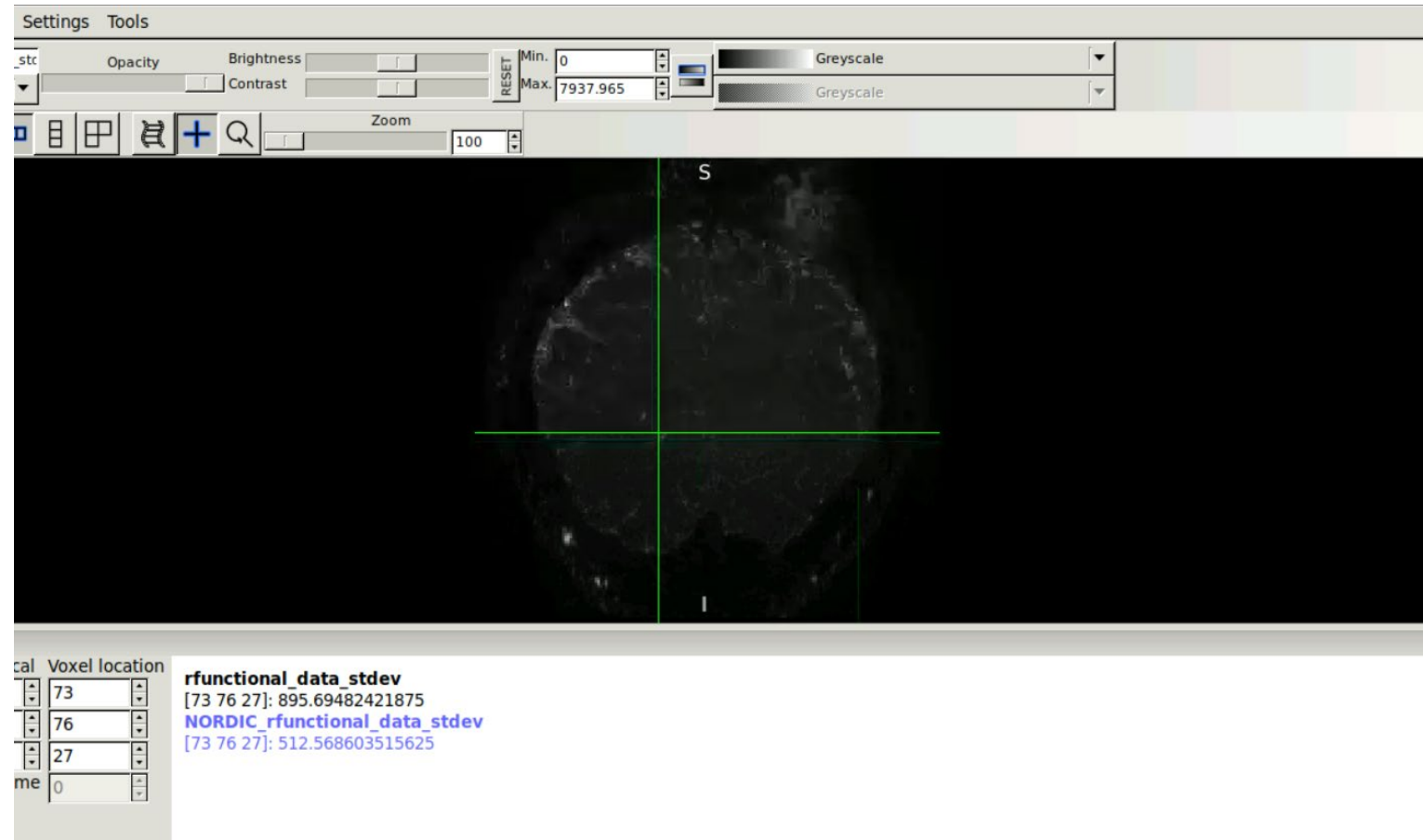
Quality Check (GLM result, no NORDIC, ONLY BOLD)

Lots of noise and false positives!



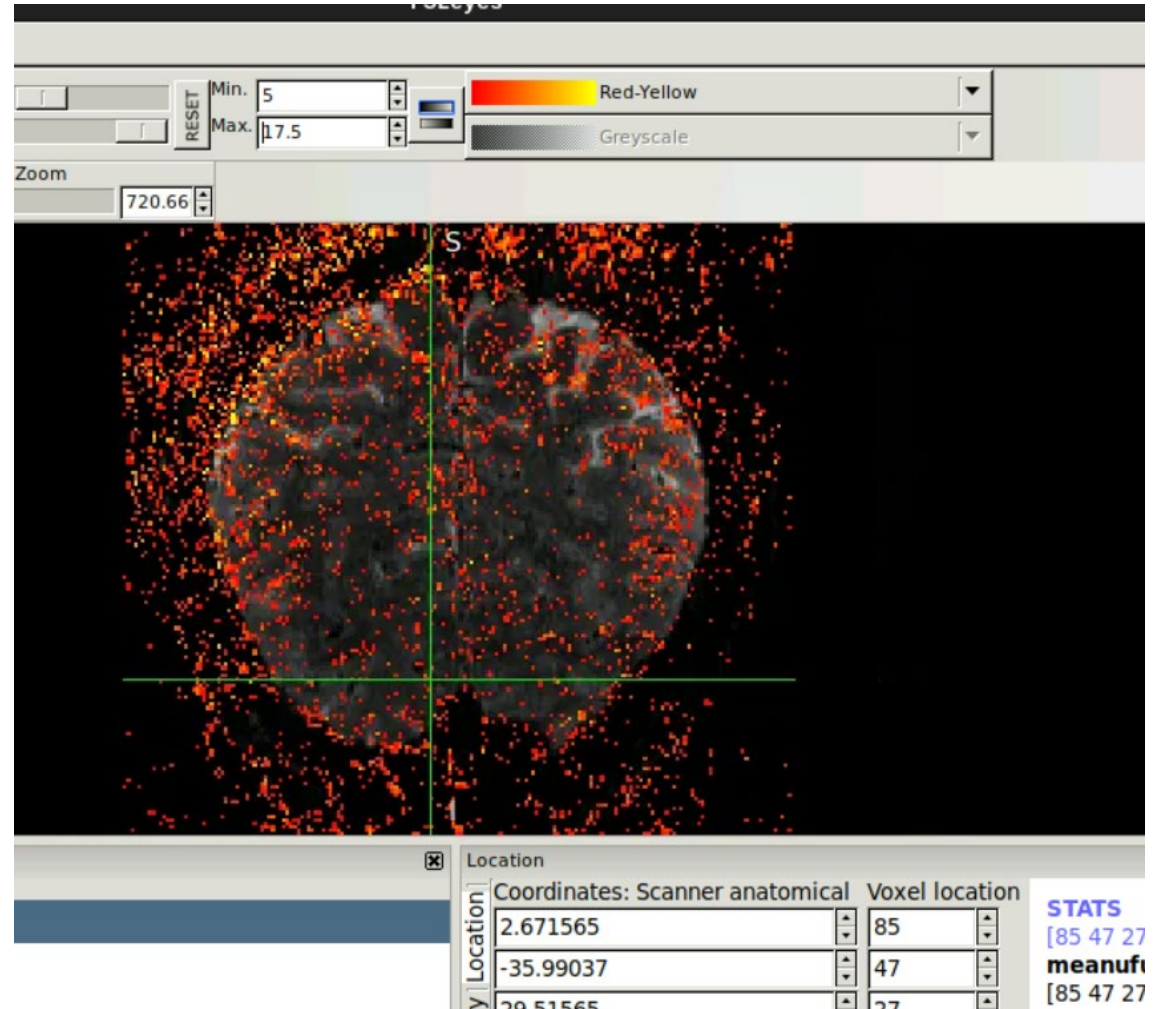
Quality Check (after NORDIC)

Overall std reduced and
tSNR improved, but we have
the ghost effect, yet.



Quality Check (after NORDIC)

I don't know why! But it seems the false positives increase!

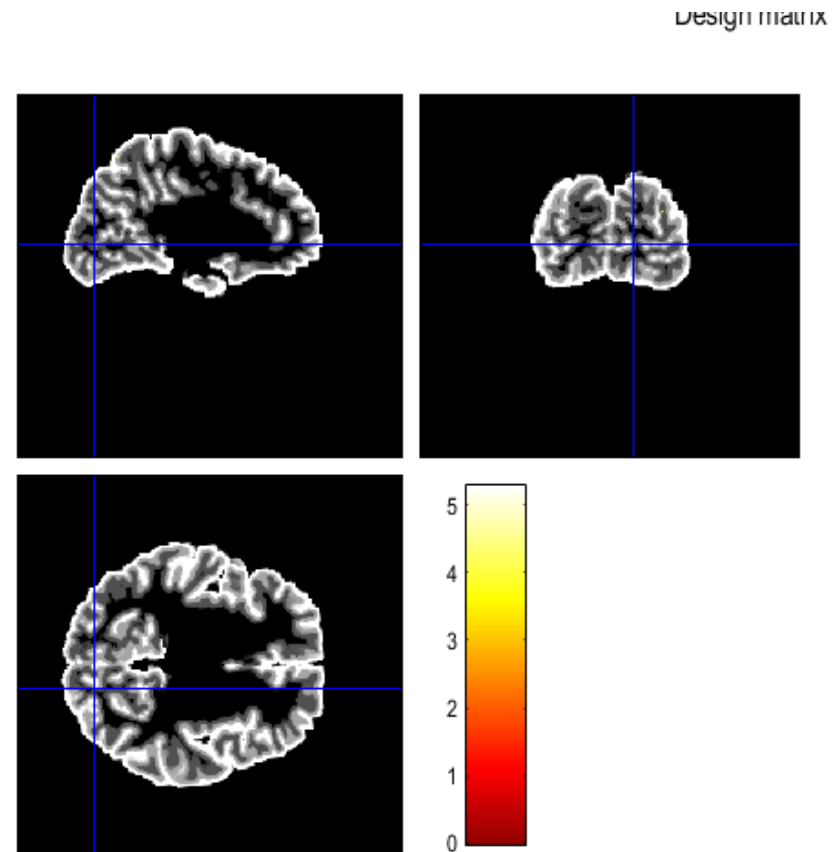


GLM results and Layers in SPM

Ori2 – Ori1 (occipital mask, whole brain)
Then I used layersbin in overly section in SPM, to see whether I could see layer activation in this way! (I am a bit blind at this stage.. Found no good resources)

I couldn't see activation in layers with SPM result.

Lets try with Afnii and FSLleys



Activation in v1 (upsampling and masking)

```
# Extract original voxel dimensions
x=$(3dinfo -di T1_weighted.nii)
y=$(3dinfo -dj T1_weighted.nii)
z=$(3dinfo -dk T1_weighted.nii)

# Calculate new voxel dimensions (e.g., upsample by a factor of 5 in x and y, keep z the same)
x=$(echo "((sqrt($delta_x * $delta_x) / 5))" | bc -l)
y=$(echo "((sqrt($delta_y * $delta_y) / 5))" | bc -l)
z=$(echo "((sqrt($delta_z * $delta_z) / 1))" | bc -l)

# Upsample the layersegmented image
3dresample -dxyz $x $y $z -rmode Cu -overwrite -prefix scaled_T1.nii -input ribbon-in-rawavg_rim_layerbins_equivol.nii

# Upsample the functional image
3dresample -dxyz $x $y $z -rmode Cu -overwrite -prefix scaled_BOLD.nii -input NORDIC_rfunctional_data.nii

# Apply V1 mask to the upsampled anatomical image
3dcalc -a ribbon-in-rawavg_rim_layerbins_equivol.nii -b v1mask.nii -expr 'a*b' -overwrite -prefix masked_scaled_T1.nii

# Apply V1 mask to the upsampled functional image
3dcalc -a NORDIC_rfunctional_data.nii -b v1mask.nii -expr 'a*b' -overwrite -prefix masked_scaled_BOLD.nii
```

Activation in v1 Layers

```
Code : LN2_PROFILE -input masked_scaled_BOLD.nii -layers  
masked_scaled_T1.nii -plot -output layer_profile_cond1.txt
```

Unfortunately, this doesn't work properly on this dataset, I guess due to poor co-registration. Dr ke jia noted about the problem of co-registering this data, and I guess my co-registration step was wrong! But the code works well in other datasets.